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Alginate–Pectin–Poly-L-lysine Particulate as a Potential Controlled Release Formulation

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Abstract

Drug delivery particulates were prepared using alginate, polylysine and pectin. Theophylline, chlorothiazide and indomethacin were used as the model drugs for in-vitro assessments, and mannitol was the model for assessing paracellular drug absorption across Caco-2 cell monolayers. Alginate and pectin served as the core polymers and polylysine helped to strengthen the particulates. Use of pectin specially helped in forming a more robust particulate that was more resistant in acidic pH and modulated the release profiles of the encapsulated model drugs in the alkaline pH. Alginate and pectin were also found to enhance the paracellular absorption of mannitol across Caco-2 cell monolayers by about three times. The release rate could be described as a first-order or square-root time process depending on the drug load.

Use of alginate–polylysine–pectin particulates is expected to combine the advantages of bioadhesion, absorption enhancement, and sustained release. This particulate system may have potential use as a carrier for drugs that are poorly absorbed after oral administration.

Advancements in drug delivery could come from innovative improvements to the existing drug delivery systems. The concept of combining the advantages of particulate and bioadhesive technologies into one delivery system is attractive because of its potential capability to deliver peptide and protein drugs (Ponchel et al 1997). While the bioadhesive part of the formulation could enhance peptide delivery by prolonging its retention, thereby localizing the drug at the site of absorption and intensifying the concentration gradient (Lehr 1994), the particulate part of the formulation could act as a carrier for the drug and protect it from the harsh physiological environment in the gastrointestinal tract. Hydrophilic polyionic polymers such as alginates and chitosan have been used for the preparation of particulates (Bodmeier & Paeratakul 1989; Østberg et al 1994; Acikgoz et al 1995). Since the process to prepare particulates made from these materials involves the use of aqueous solvents, stability-related (Cleland & Jones 1996), toxicological, and environmental problems associated with organic solvents would be minimized. These particulates would also have bioadhesive properties (Smart et al 1984).

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In this work we have used alginate and pectin as the core materials for preparing drug-loaded particulates. Alginates are anionic polysaccharides derived from brown algae and comprise D-mannuronic and L-guluronic acid residues joined linearly by 1,4 glycosidic linkages. A solution of sodium alginate upon contact with calcium ions in aqueous solution forms a hydrogel. This is a complex coacervation process occurring due to the physical cross-linking between carboxylate anions of guluronate and calcium ions. Although the hydrogel structure of calcium alginate is labile in the presence of monovalent cations, the gel structure could be strengthened by using different compositions of alginate, calcium ion concentrations, or gelation time (Bodmeier & Wang 1993; Kikuchi & Wang 1997). However, attempts to prolong the release of conventional drugs using alginate particulates beyond a few hours have improved release profiles only marginally, despite the use of processes that involve heating (Bodmeier & Wang 1993) or the use of other polymers such as chitosan (Murata et al 1996). The difficulty in sustaining drug release is more for aqueous soluble drugs and those with smaller molecular weights, such as theophylline, acetaminophen and chloramphenicol (Østberg et al 1994). Attempts to strengthen alginate particulates by complexing with

cationic chitosan (Murata et al 1996) or polylysine (Thu et al 1996) have had varying degrees of success.

We have developed a particulate carrier system which consists of alginate and pectin beads coated with polylysine. Pectin is a heterogenous polysaccharide, consisting mainly of D-galacturonic acid and its methyl ester linked by α (1–4) glycosidic linkage, and is present in the cell wall of most plants. It also forms a gel in the presence of calcium ions, but the gel is more resistant to breakage in the gut than alginate gel, breaking down in the presence of micro flora in the colon, and has been used for colonic drug delivery (Ashford et al 1993, 1994). In our particulates the presence of pectin as an additional core material allowed controlled release of small molecular weight drugs. The preparation procedure was simple and would be easily scalable. The model drugs chosen for the in-vitro experiments were theophylline, chlorothiazide and indomethacin. The ability of the particulate ingredients, alginate and pectin, to improve drug absorption across Caco-2 cell monolayers was examined using mannitol as a model compound.

Materials and Methods

Materials

Sodium alginate (suppliers' specification: viscosity of 2% solution at 25°C, 3500 cps), poly-L-lysine hydrochloride (MW: ~22 000 Da), pectin as a potassium salt (degree of esterification: 28%), calcium chloride, glass beads (1·2 mm), mannitol, [^{14}C]mannitol (sp. act.: 50 mCi mmol^{-1}), theophylline, chlorothiazide, indomethacin and Dulbecco's Modified Eagle's Medium (DMEM, with 4·5 g L^{-1} glucose, 10% foetal calf serum, 1% nonessential amino acids and antibiotics) were all purchased from Sigma Chemicals, MO, USA. Foetal calf serum, penicillin–streptomycin and sodium bicarbonate were obtained from Gibco BRL, Life Technologies (NY, USA). Sprague–Dawley rats, 220–320 g (Animal Care Center, Memorial University of Newfoundland, NF, Canada), were used for obtaining parts of the gastrointestinal tract for bioadhesive experiments. The Caco-2 cell line was obtained from American Type Culture Collection (MD, USA).

Preparation of drug-loaded calcium alginate particulates

The method to prepare sodium alginate particulates was adapted from Bodmeier & Paeratakul (1989). A solution of sodium alginate (1·5% w/v) was prepared in deionized water. The model drug was dispersed in the alginate solution using a homogenizer (Brinkmann Instruments Company, Switzerland; 1000 rev min^{-1}). The dispersion was added drop-wise using a disposable syringe (21 gauge), into a gently agitated aqueous solution of calcium chloride (2%, w/v). Although the gelation process began almost instantaneously, different gelation times were studied to determine optimum preparation conditions. The particulates were harvested and rinsed with deionized water to remove loosely adhered drug. The particulates were then freeze-dried (Labconco Corp., MO, USA) without any pre-cooling. Remaining traces of moisture were removed by desiccating under vacuum for 48 h. A few test samples were also air-dried for comparison with the freeze-dried products.>

Preparation of alginate particulates coated with polylysine

The procedure was essentially similar to the one described above with an additional step where the alginate particulates were coated with a 0·1% w/v solution of polylysine. Essentially the particulates formed in the calcium chloride solution were suspended in polylysine solution for 6 min to allow cross-linking to occur between alginate (anionic) and polylysine (cationic). The particulates were filtered out, washed with deionized water and suspended in sodium alginate solution (0·05% w/v) to neutralize any excess polylysine at the surface. These particulates were finally dried as described above.

Preparation of alginate–pectin particulates coated with polylysine

These particulates were prepared as described above with a slight modification. In this procedure, 3·7% (w/v) pectin was mixed along with the model drug and dispersed in sodium alginate solution.

Particle size and scanning electron microscopy

Particle size was determined using a computer image analyser (Bioquant system IV, USA). The particles were observed both in dry form as well as after allowing them to swell in the buffer (pH 6·0) for 10 min.

For the scanning electron microscopy (Hitachi S570 SEM, Japan), cross-sections of the particles were obtained using a razor blade. The sections were then coated with gold–palladium for 70 s in an argon atmosphere before observing them under the microscope.

In-vitro release studies

The release studies were performed using a USP Type I (basket) dissolution apparatus (Vanderkamp 600, Vankel Industries Inc., NJ, USA) attached to a fraction collector (Vanderkamp model 10, Vankel Industries Inc., NJ, USA). The stirrer speed was set to 120 rev min^{-1} and the thermostat-controlled bath was set to 37°C. The dissolution fluids (900 mL) consisted of 0·1 M HCl and phosphate buffer (0·05 M, pH 7·5). Drug-loaded particulates weighing between 40 and 100 mg (depending on the type of model drug) were placed in the basket. Five millilitres of dissolution fluid was sampled at predetermined time intervals. The sample volume was replenished with fresh dissolution fluid. The amount of drug released was quantified spectrophotometrically (theophylline, $\lambda_{max} = 272$ nm; chlorothiazide, $\lambda_{max} = 294$ nm; indomethacin, $\lambda_{max} = 266$ nm) from the standard curves prepared for each model drug. All experiments were performed in triplicate and the mean cumulative % of drug released ± s.d. are reported.

The drug load was determined by calculating the cumulative amount of the drug dissolved in phosphate buffer (pH 7·5) after 72 h of dissolution study. All three particulates, alginate, alginate–polylysine and alginate–pectin–polylysine, were observed to completely disintegrate after 48 h of dissolution and hence, 72 h was considered as the adequate time period to release all the encapsulated drugs.

Determination of bioadhesive strength

This procedure was adapted from Rao & Buri (1989). Unfasted male Sprague–Dawley rats (300–350 g) were killed using an overdose of urethane. The stomach and jejunum were cut open, emptied of food and washed with 0·1 M HCl (20 mL min^{-1}) and phosphate buffer (pH 6·0, 20 mL min^{-1}), respectively, until they were clean. The gut pieces were used as the bio-surface to test the bioadhesive strength of the test particulates. Cleaned sections of the gut were used within 2 h. Pieces (5–7 cm) of jejunum or stomach were cut and placed on one half of a longitudinally cut rubber tube (2 cm diam.) with the help of pins. Silica-coated glass beads were used as the control samples. About 50 mg of test samples were placed on the piece of tissue (stomach or jejunum) and incubated for 20 min in an 80% r.h. chamber, prepared by saturating the chamber with a saturated solution of ammonium chloride at room temperature (25 ± 1°C). This procedure allowed the particulates to hydrate and interact with the mucosal surface of the gut. Then the tissue-particulate assembly was placed on the plastic support fixed at an angle of 45°. A rubber tube connected to a peristaltic pump was placed about 1 cm above the tissue sample. The particulates were washed by pumping either dilute HCl or phosphate buffer solution at 25 mL min^{-1} for 1 h. The particulates retained on the gut tissue were quantified, which reflected their bioadhesive property.

Transport studies using Caco-2 cell monolayers

For mannitol transport experiments, Caco-2 cells (passages 26–29) were cultivated on polycarbonate filters (Costar Transwell 6-well plate inserts, pore size 0·4 μm, Costar, MA, USA) at a seeding density of 3×10^5 cells/well. After 21–23 days of seeding, the cells were suitable for conducting the transport study. DMEM (with 4·5 g L^{-1} glucose) supplemented with 10% foetal calf serum, 1% non-essential amino acid solution, 2% sodium bicarbonate, benzylpenicillin G (50 int. units mL^{-1}), and streptomycin sulphate (50 μg mL^{-1}) was used as the culture medium. The medium was added to both the donor and the acceptor compartments, which was changed every other day. The cell cultures were incubated at 37°C, in an atmosphere of 5% CO_2 and 95% air. To a solution of 1% w/v sodium alginate in DMEM, pectin was added and dissolved to obtain 1% and 2% (w/v) solutions: addition of pectin beyond 2% resulted in a very viscous solution that was difficult to handle. The model compound, mannitol, was dissolved in this solution to obtain 0·1% (w/v) solution. Radiolabelled [^{14}C]mannitol (sp. act. 0·25 μCi mL^{-1}) was added as a tracer to facilitate analysis by liquid scintillation counting (Beckman LS 5000TD, CA, USA). The control solution consisted of 0·1% mannitol and the tracer, dissolved in DMEM. Samples (100 μL) were taken every 30 min for 3 h from the basolateral side. The volume removed from the basolateral side was replenished with fresh DMEM. Samples were also taken from the stock solutions to determine total radioactivity. To each of the test samples, 5 mL scintillation fluid (Formula 989, Dupont NEN Research Products, MA, USA) was added before determining the disintegrations per minute (d min^{-1}) using the scintillation counter. The background radioactivity was determined by using 100 μL DMEM as the test sample. All experiments were carried out in triplicate and the mean ± s.d. are reported.

The apparent permeability coefficients (P_{app}, cm s^{-1}) were determined using the following equation (Artursson 1990):

$$P_{app} = \Delta Q/\Delta t \times 60 \times A \times C_0 \qquad (1)$$

where $\Delta Q/\Delta t$ is the permeability rate (d min^{-1} min^{-1}), C_0 is the initial concentration of mannitol in the donor chamber (d min^{-1} mL^{-1}) and A is the surface area of the membrane (4·71 cm^2).

Results and Discussion

The important features of a good drug delivery system include versatility to carry drugs with different physicochemical properties, simplicity of the method of preparation, and feasibility for mass production. We have attempted to bear these factors in mind while formulating the particulates. With the use of organic solvents for preparing particulates such as polylactide glycolide microspheres, there are two main problems: toxicity due to the residual organic solvents and instability of certain drugs, especially those belonging to the class of peptides and proteins (Cleland & Jones 1996). Our method for preparing drug-loaded particulates involves only aqueous solvents. Although at this initial stage of development we have used conventional drug molecules as model compounds, the technique is also expected to be applicable to peptide and drug proteins.

Particle size and scanning electron microscopy

The particulates were produced using a 21-gauge hypodermic syringe, smaller sized particles could be prepared by using compressed air to force the droplets through a narrower syringe (Kwok et al 1991). The drying technique did influence the final shape and size of the particulates. We observed that freeze-dried particulates were 1·5–2 mm in diameter and spherical in shape. The air-dried particulates were 0·5–1 mm in diameter and irregular in shape. Both swelled up to about the same size (approx. 3 mm) when placed in an aqueous solution. Freeze-dried formulations would be preferred as they would protect thermolabile drugs. We used freeze-dried particulates in all our experiments.

The cross-sections of freeze-dried particulates viewed under a scanning electron microscope (SEM) showed numerous, honeycomb-like open cavities (Figure 1b, c, d), as opposed to a less open and denser structure for the air-dried ones (Figure 1a). Although both sodium alginate and pectin are known to form hydrogels upon contact with calcium ions (Bodmeier & Paeratakul 1991; Ashford et al 1994), we observed that the ionotropic gelation was spontaneous for alginate while that for pectin took some time and the particulates did not assume a spherical shape. Formation of alginate–pectin–polylysine particulates was spontaneous, similar to the formation of alginate and alginate–polylysine particulates and the shape remained spherical. Hence, it seems that the presence of alginate is very much essential for spontaneity of ionotropic gelation and for the maintenance of a

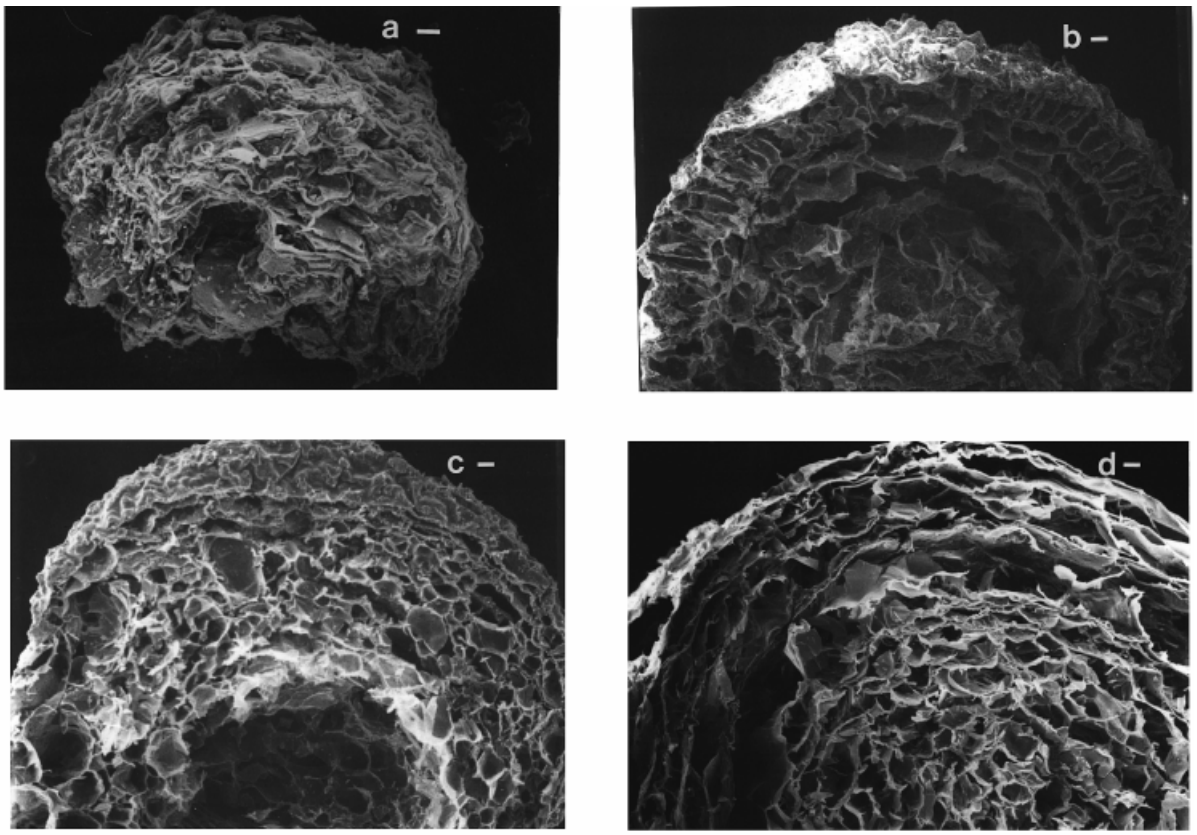

Figure 1. Scanning electron micrographs of the cross-sections of particulates: a, air-dried alginate particulates; b, freeze-dried alginate particulates; c, freeze-dried alginate–polylysine particulates; d, freeze-dried alginate–pectin–polylysine particulates. Bar = 60 μm.

spherical shape of the particle. Besides, being polyanionic alginate also served as a template to the polycation, polylysine, which helped in prolonging the life of alginate particulates in aqueous solutions. Although the concentration of polylysine in the particulates was low, a final coating of sodium alginate was added to neutralize any unreacted polylysine. This would minimize any unwanted immune responses to polylysine, and would also provide a negative surface charge to the particulates that would allow them to bind to mucous membranes (Thu et al 1996). Pectin was used as the co-core material. Its ability to withstand degradation in gastric pH made the particles more robust. As a result, the particulate's ability to sustain drug release was significantly improved.

Drug loading and gelation time

Drug loading depends on the solubility of the drug and the gelation time allowed. Although we did not perform a detailed study to see the effect of gelation on the release profile, we did compare theophylline releases in 0·1 M HCl and phosphate buffer from alginate–pectin–polylysine particulates with 10 and 20 min gelation times. The dissolution times for releasing 90% (t90) in 0·1 M HCl were 4 and 6 h for the 10 and 20 min gelation times, respectively. In the alkaline medium the t90 values were 2 and 3·5 h, for the 10 and 20 min gelation times, respectively. In principle, this is in agreement with the results reported by Bodmeier & Wang (1993), an increase in gelation time resulted in prolonging the drug release. This is possibly due to the formation of a gel structure with a greater number of cross-linkings. But longer gelation time would also lead to leaching of soluble drugs from the particulates to the surrounding aqueous medium, thereby reducing drug loading. We chose 20 min as the optimum gelation time for all our experiments. The drug loadings were 23–26% for theophylline, 45% for chlorothiazide and 47% for indomethacin in all the particulates prepared.

In-vitro release profiles of drugs from particulates

The model drugs chosen for the in-vitro experiments, theophylline, chlorothiazide and

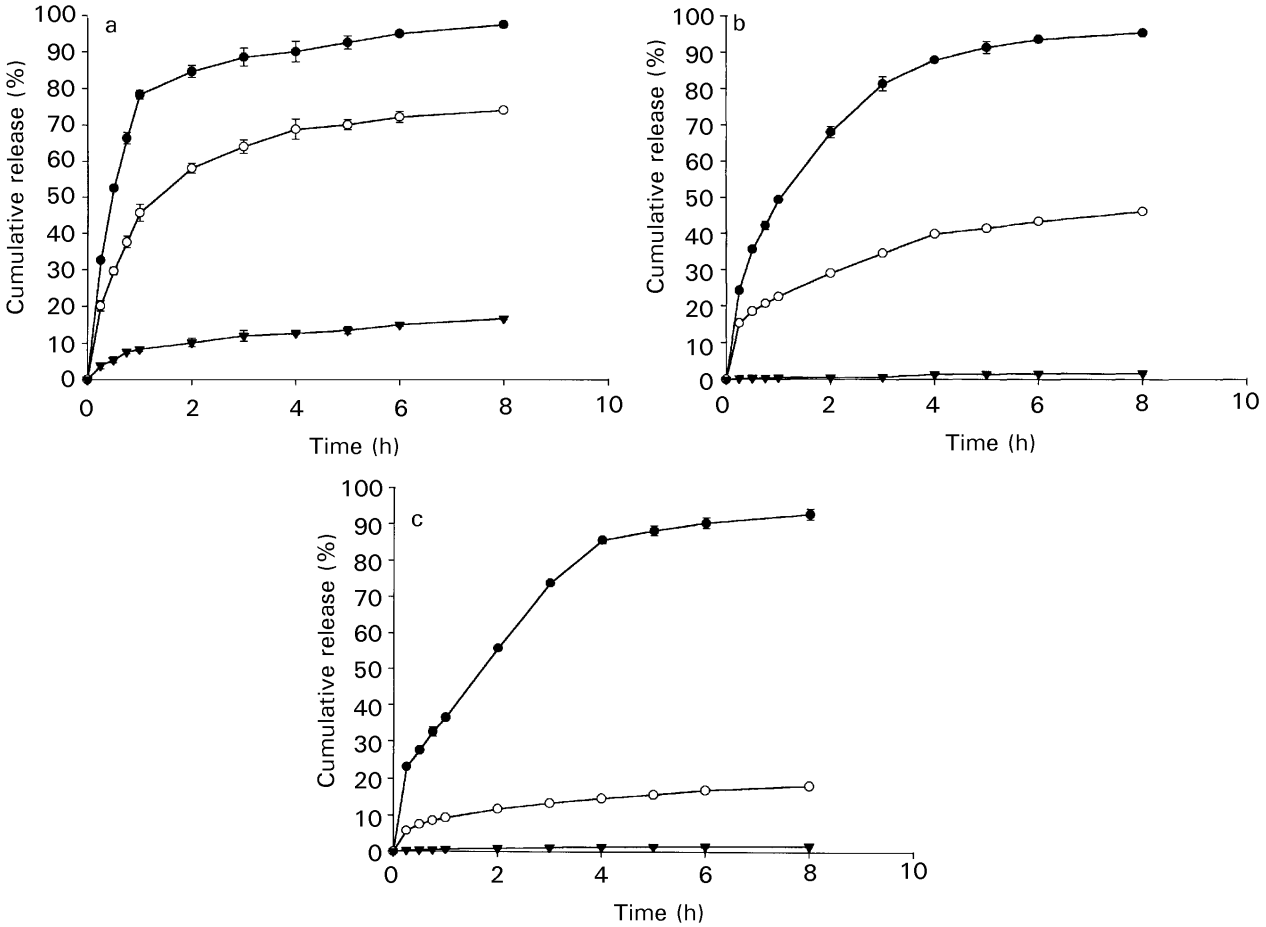

Figure 2. Cumulative release profiles of model drugs in 0·1 M HCl from (a) alginate particulates, (b) alginate–polylysine particulates and (c) alginate–pectin–polylysine particulates. The model drugs are: ● theophylline; ○ chlorothiazide; ▼ indomethacin. Values are mean ± s.d.

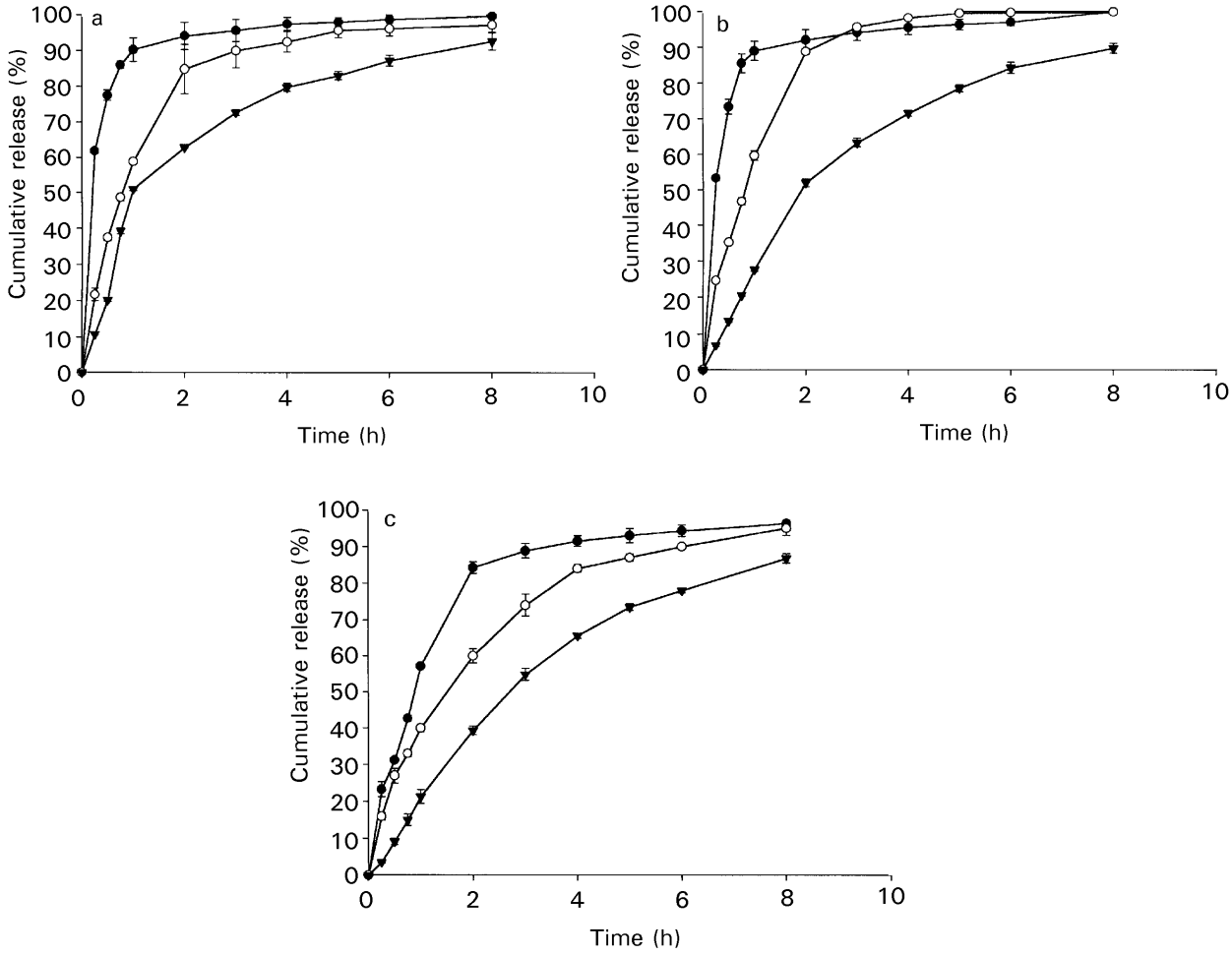

Figure 3. Cumulative release profiles of model drugs in phosphate buffer pH 7·5 from (a) alginate particulates, (b) alginate–polylysine particulates, and (c) alginate–pectin–polylysine particulates. The model drugs are: ● theophylline; ○ chlorothiazide; ▼ indomethacin. Values are mean ± s.d.

indomethacin, represent the wide range of conventional drugs generally in use. Theophylline is relatively soluble (8·33 g L^{-1}), chlorothiazide is very slightly soluble (0·4 g L^{-1} at pH 4 and 0·65 g L^{-1} at pH 7) and indomethacin is practically insoluble in water (The Merck Index 1996). The drug release profiles were studied at two pH conditions, acidic (0·1 M HCl, Figure 2) simulating the gastric condition, and pH 7·5 (Figure 3) simulating the intestinal condition. The dissolution times for releasing 50% and 90%, i.e. t50 and t90, are given in Table 1.

Table 1. Dissolution half-life (t50) and t90 of the particulates in acid and alkaline media.

Model drug	t50 (h)			t90 (h)		
	Alginate	Alginate–polylysine	Alginate–pectin–polylysine	Alginate	Alginate–polylysine	Alginate–pectin–polylysine
In 0·1 M HCl						
Theophylline	0·49 ± 0·008	1·05 ± 0·004	1·70 ± 0·008	3·91 ± 0·028	4·60 ± 0·017	6·05 ± 0·015
Chlorothiazide	1·38 ± 0·021	–	–	–	–	–
Indomethacin	–	–	–	–	–	–
In phosphate buffer pH 7·5						
Theophylline	0·21 ± 0·007	0·24 ± 0·008	0·90 ± 0·007	0·96 ± 0·033	1·32 ± 0·028	3·50 ± 0·015
Chlorothiazide	0·80 ± 0·009	0·82 ± 0·012	1·50 ± 0·015	3·00 ± 0·048	2·20 ± 0·008	6·02 ± 0·04
Indomethacin	1·00 ± 0·004	1·92 ± 0·011	2·70 ± 0·016	7·02 ± 0·023	8·10 ± 0·014	9·21 ± 0·013

Values are mean ± s.d.

Release in acidic solution

In acidic solution only the very soluble drug, theophylline was released from all the three particulates (Figure 2). The t50 values for theophylline in acidic solution were 0·49 h for alginate, 1·05 h for alginate–polylysine and 1·70 h for alginate–pectin–polylysine. The t90 values also showed a similar trend (Table 1).

Although chlorothiazide has a finite solubility in water, it was released only from the alginate particulates in 0·1 M HCl (t50 1·38 h). An insufficient amount of chlorothiazide was released from the alginate–polylysine and alginate–pectin–polylysine particulates to enable determination of its t50 value. Similarly, the t90 values of chlorothiazide could not be determined for any of the particulates in 0·1 M HCl during the 8-h study. For the least soluble drug indomethacin, the amount of drug released from the three particulates was far less than their t50 and t90 values.

Thus it may be concluded that drug release from the particulates would be minimal in acidic conditions, such as in the stomach. It is also evident that drug release would be slowest from the alginate–pectin–polylysine particulates.

Release in alkaline solution

Release of the drug molecules in alkaline pH 7·5 was gradual. For theophylline, t50 values from alginate (Figure 3a), alginate–polylysine (Figure 3b) and alginate–pectin–polylysine (Figure 3c) particulates were 0·21, 0·24 and 0·9 h, respectively, and the t90 values were 0·96, 1·32 and 3·50 h, respectively (Table 1). Plain alginate particulates, although relatively stable in acidic conditions, were unable to control the release of the very soluble drug, theophylline beyond 1 h. Coating with polylysine helped only marginally in prolonging the release of theophylline. The most significant difference was obtained with alginate–pectin–polylysine particulates where the t90 was 3·5 h. This could be attributed to the presence of pectin gel which is stronger and more stable than alginate gel in acidic and alkaline solutions.

Multivalent ions such as calcium, exchange with sodium ions of sodium alginate solution to form calcium alginate gel. On placing the gel in an ionic solution the reverse process takes place, resulting in the gel-to-sol transformation. Contradictory results are reported in the literature with regard to the dissolution rate of alginate particulates in the acid medium. While Bodmeier & Paeratakul (1989) have reported a slower release of drug from calcium alginate particulates in acidic solution than in alkaline, Østberg et al (1994) have reported a faster release in acidic solution. Our observation was similar to results reported by Bodmeier & Paeratakul (1989). This could be attributed to the differences in the chemical composition in the alginate used by the different groups. Commercially available alginate comes in various grades that differ in the guluronic/mannuronic acid ratios and the level of free acid functions (Timmins et al 1992). Since the alginates used by us and Bodmeier & Paeratakul (1989) were similar and obtained from the same supplier, it is assumed that they had a similar chemical composition and hence, similar physical properties. Østberg et al (1994) used alginate from a different source (Pronova Biopolymer, Drammen, Norway, MW 200 000–270 000 Da) which had a different composition from that of ours.

Mechanism of drug release and release kinetics

The mechanism of release in all the particulates would be a combination of diffusion and erosion (Timmins et al 1992). Drug release from intact particulates would be predominantly by diffusion. Alginate particulates being more labile are expected to be prone to erosion. The cross-linking in calcium alginate gel gets destroyed as the Ca^{2+} ions are exchanged in the presence of monovalent cations. Although morphologically there may not be a significant change in the swelling characteristics of the matrices, the gel structure that retards drug release gets destroyed by the loss of Ca^{2+} ions (Østberg et al 1994). The alginate–pectin–polylysine particulates with a rigid pectin gel inside would be expected to resist erosion and prolong drug release. We chose a pectin with a low degree of methoxylation (28%) that was expected to be more soluble in water and able to form a gel with calcium ions relatively easily (Ashford et al 1994). It should be possible to choose alginate and pectin with different chemical compositions to custom design the release profile desired for a particular drug.

To elucidate the kinetics of drug release from the particulates the percentage of drug remaining to be released (Q) was plotted as a function of time (t) in the following equations:

$$Q = k_a t \qquad \text{zero-order equation} \qquad (2)$$

$$\ln Q = kt \qquad \text{first-order equation} \qquad (3)$$

$$Q = kt^{1/2} \qquad \text{square root-time equation} \qquad (4)$$

Linear regression analyses were performed for the three equations of straight lines and their correlation coefficients (r^2) were determined (Table 2). The release rate could be described as following a first-order or square root-time process depending on

Table 2. Correlation coefficients (r^2) calculated using different kinetic orders of drug release from particulates.

Model drug	Zero-order			First-order			$t_{1/2}$		
	Alginate	Alginate–Polylysine	Alginate–pectin–polylysine	Alginate	Alginate–Polylysine	Alginate–pectin–polylysine	Alginate	Alginate–Polylysine	Alginate–pectin–polylysine
In 0·1 M HCl									
Theophylline	0·55	0·77	0·84	0·92	0·97	0·96	0·79	0·95	0·96
Chlorothiazide	0·71	0·82	0·81	0·84	0·88	0·83	0·91	0·97	0·96
Indomethacin	0·84	0·91	0·89	0·86	0·91	0·89	0·97	0·91	0·99
In phosphate buffer pH 7·5									
Theophylline	0·55	0·77	0·84	0·92	0·97	0·96	0·79	0·95	0·96
Chlorothiazide	0·71	0·82	0·81	0·84	0·88	0·83	0·91	0·97	0·96
Indomethacin	0·84	0·91	0·89	0·86	0·91	0·89	0·97	0·91	0·99

the drug load. For theophylline particulates (drug load 23–26%) the release rates were closer to a first-order process both in acid and alkaline solutions. For chlorothiazide (drug load 45%) and indomethacin (drug load 47%) particulates the amount of drug released in the acidic medium was proportional to the square root of time. Their release in the alkaline medium could be described either as a first-order or square root-time process. This is in general agreement with the release mechanisms described for reservoir systems without a rate controlling membrane, in which there is an initial burst followed by a slower release that is proportional to the square root of the time (Baker 1987).

In-vitro bioadhesive strength

The Rao & Buri (1989) method was used to estimate the bioadhesive strengths of the particulates. All of the three particulates adhered to the stomach and intestinal tissue (100% adherence), and did not get washed-out. The control glass beads had no bioadhesive property (0% adherence) and were washed-out within the first 5 min of elution. Although the test was able to demonstrate the presence of the bioadhesive property for all the particulates, it was not quantitative enough to differentiate between their relative adhesive strengths. Despite elution for 1 h all the polymeric particulates remained adhered to the mucosal tissues. This shows that the particulates did not erode under the conditions of the test, which would have otherwise resulted in a reduction in bioadhesion with progression of time.

Transport study

Use of Caco-2 cell monolayers for an in-vitro estimation of the extent of drug absorption from an oral dosage form is well documented (Artursson 1990; Schipper et al 1996; Brayden 1997). Mannitol is typically used as the paracellular tight-junctional marker (Brayden 1997). One of the limitations of the study is that the test drug substance has to be in solution. Our test sample consisted of DMEM solution containing different concentrations of sodium alginate and pectin, with 0·1% w/v mannitol (plus [^{14}C]mannitol as the tracer) as the model marker. DMEM containing only 1% mannitol served as the control. The apparent permeability coefficients (P_{app}) are given in Figure 4. For the control sample the P_{app} gradually increased and reached a plateau value of $1{\cdot}1 \times 10^7\,cm\,s^{-1}$ in 90 min. For both the test samples there were significant enhancements in the apparent permeabilities of mannitol. Both test solutions contained 1% alginate but differed in their pectin content (1% and 2% w/v). In 30 min, the test sample with 1% pectin reached 87% peak P_{app} value ($3{\cdot}0 \times 10^7\,cm\,s^{-1}$) whereas, the test sample with 2% pectin reached its peak P_{app} ($3{\cdot}1 \times 10^7\,cm\,s^{-1}$). There was no statistical difference ($P > 0{\cdot}05$) in their plateau P_{app} (range 2·9–$3{\cdot}0 \times 10^7\,cm\,s^{-1}$) which was attained in about 60 min. The threefold increase in P_{app} of mannitol, compared with control, across epithelial cells in the presence of alginate and pectin could be of significant importance in improving oral drug delivery of poorly absorbed drugs. We would like to point out that the actual dosage form, drug-loaded alginate–pectin particulates, will also have bioadhesive property. This would mean that these particulates could be expected to adhere to the mucosal sites in the gut. It is generally believed that achieving an absorption enhancing effect quickly, with the simultaneous presence of high drug concentration at the site, is difficult to achieve (De Boer & Breimer 1994). In our study, since the permeability enhancing effect of alginate–pectin was observed within 30 min of contact with Caco-2 cells, it would be reasonable to expect that our

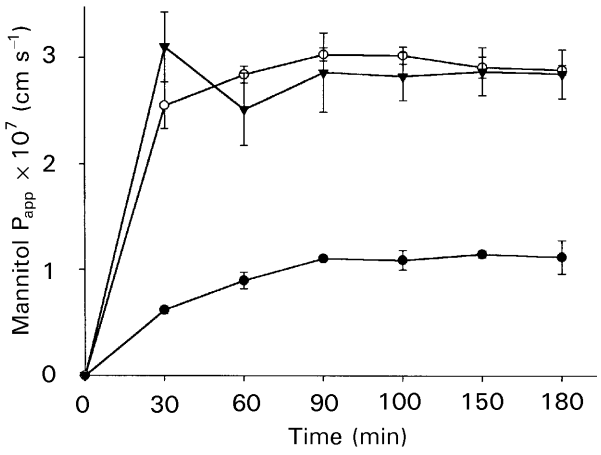

Figure 4. Transport of mannitol (expressed as P_{app} as a function of time) from alginate and pectin solution across Caco-2 cell monolayers. Mannitol was dissolved in Dulbecco's medium (control, ●), Dulbecco's medium containing 1% alginate and 1% pectin (○) and Dulbecco's medium containing 1% alginate and 2% pectin (▼). Values are mean ± s.d.

drug-loaded particulates would have the combined advantages of enhanced permeability and increased drug concentration at the absorption site. Thus the alginate–pectin–polylysine particulates seem to have the potential to be used as carriers for drugs that are poorly absorbed when administered orally. Also, although our study used conventional drug molecules as model compounds, the technique is expected to be applicable to peptide and protein drugs.

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